

G Protein Diversity: A Distinct Class of {alpha} Subunits is Present in Vertebrates and Invertebrates

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G protein diversity: A distinct class of α subunits is present in vertebrates and invertebrates

(GTP-binding protein/signal transduction/*Drosophila*)

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ABSTRACT Heterotrimeric guanine nucleotide-binding proteins (G proteins) are integral to the signal transduction pathways that mediate the cell's response to many hormones, neuromodulators, and a variety of other ligands. While many signaling processes are guanine nucleotide dependent, the precise coupling between a variety of receptors, G proteins, and effectors remains obscure. We found that the family of genes that encode the α subunits of heterotrimeric G proteins is much larger than had previously been supposed. These novel alpha subunits could account for some of the diverse activities attributed to G proteins. We have now obtained cDNA clones encoding two murine α subunits, $G\alpha_q$ and $G\alpha_{11}$, that are 88% identical. They lack the site that is ordinarily modified by pertussis toxin and their sequences vary from the canonical Gly-Ala-Gly-Glu-Ser (GAGES) amino acid sequence found in most other G protein α subunits. Multiple mRNAs as large as 7.5 kilobases hybridize to $G\alpha_q$ specific probes and are expressed at various levels in many different tissues. $G\alpha_{11}$ is encoded by a single 4.0-kilobase message which is expressed ubiquitously. Amino acid sequence comparisons suggest that $G\alpha_q$ and $G\alpha_{11}$ represent a third class of α subunits. A member of this class was found in *Drosophila melanogaster*. This α subunit, $DG\alpha_q$, is 76% identical to $G\alpha_q$. The presence of the G_q class in both vertebrates and invertebrates points to a role that is central to signal transduction in multicellular organisms. We suggest that these α subunits may be involved in pertussis toxin-insensitive pathways coupled to phospholipase C.

The G proteins are a family of guanine nucleotide-binding proteins that relay signals from cell surface receptors to intracellular effectors. Members of this family are heterotrimers composed of α , β , and γ subunits. The α subunit is believed to confer receptor and effector specificity on the heterotrimer. When the G protein is activated by interaction with receptor, the α subunit exchanges bound GDP for GTP. The intrinsic GTPase activity of the α subunit restores it to the basal state in which GDP is bound. This form of signal transduction is basic to the mechanisms that cells use in responding to hormones, neurotransmitters, and a variety of other ligands (for reviews see refs. 1–3). The process is highly conserved in evolution. Indeed, G proteins are central to intercellular communication among even simple eukaryotes. For example, G proteins are involved in the yeast mating-type pathway (4, 5), and several G protein α subunits are differentially expressed during development in the slime mold *Dictyostelium discoideum* (6, 7).

We are interested in how G protein-mediated signal transduction has adapted to the diverse signaling requirements of complex multicellular organisms. Reconstitution studies and the use of pertussis and cholera toxins to modify specific G protein α subunits have demonstrated the involvement of the

$G\alpha_s$ and $G\alpha_i$ subtypes in gating of specific ion channels (8, 9) and in the regulation of adenylyl cyclase in a variety of organisms (10). In the highly specialized visual system in mammals, biochemical experiments have led to the elucidation of the role of $G\alpha_{11}$ (rod transducin) in regulating phosphodiesterase and subsequently in controlling the levels of cyclic GMP (11). There are, however, many processes that are refractory to toxin inhibition but nonetheless appear to be mediated by guanine nucleotide-binding proteins (12, 13). To understand the extent of involvement of the G protein system and the nature of the specificity required for function, we have examined the diversity of the G protein family in complex organisms.

Recently, we developed an approach involving the polymerase chain reaction (PCR, ref. 14) to detect novel sequences that share highly conserved domains common to all G protein α subunits. We found evidence for extensive diversity in the mammalian G protein α subunit family (15). A small screen uncovered five novel sequences termed $G\alpha_{10}$ through $G\alpha_{14}$. In this paper we present the cDNA sequences of two α subunits that define another class of G proteins. This class, termed G_q , is distinguished by amino acid sequence homology and includes $G\alpha_q$, $G\alpha_{11}$, and $G\alpha_{14}$. We argue that the G_q class appeared early in evolution; it is found in both vertebrates and invertebrates. We present the sequence of a member of the G_q class in *Drosophila*.*

MATERIALS AND METHODS

PCR. PCR was performed as described previously (15). cDNA was made from poly(A)⁺ RNA with random hexanucleotide primers by using reverse transcriptase from Moloney murine leukemia virus. Conditions were those described by the supplier (BRL). The oligonucleotides used for PCR amplification of the cDNA were as follows:

oMP19, CGGATCCAARTGGATHCAYTGYYT;
oMP20, GGAATTCRTCTYTTYTTRTTNAGRAA;
oMP21, GGAATTCRTCTYTTYTTRTTYAARAA;
GQ112, CTGAAGGAGTACAACTGGT;
GQ3, GACACGAATGACAGGAGTGCT; and
G113, CCTCAAGCCACATTGAGTCA,

in which R = A or G, Y = C or T, H = A, C, or T, and N = A, C, G, or T. PCR was performed on a Perkin Elmer Cetus thermal cycler. During each cycle, samples were denatured for 1.0 min at 94°C, extended for 1.0 min at 72°C, and annealed for 0.5 min at the following temperatures: oMP19 + oMP20 + oMP21, 42°C; GQ112 + GQ3, 53°C; and GQ112 + G113, 53°C. Each oligonucleotide was used in the PCR at 10 ng/ μ l. Thirty-five cycles were performed on approximately 5

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Abbreviation: PCR, polymerase chain reaction.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M55412 for $G\alpha_q$ and M55411 for $G\alpha_{11}$).

Isolation of cDNAs. The G69 cDNA clone (Fig. 1A) was isolated from an oligo(dT)-primed mouse brain library in the λ cloning vector m λ JSM8 (unpublished results); 10^5 clones were screened at a density of 10^4 plaques per 150-mm plate. Nitrocellulose filters (Millipore) were prepared by standard techniques (ref. 16, p. 12.30). Appropriate restriction enzymes were used to excise the coding regions of cDNA clones encoding rat Ga_s , Ga_{i1} , Ga_{i2} , Ga_{i3} , and Ga_o ; bovine Ga_{i1} and Ga_{i2} ; and human Ga_7 . These cDNAs were com-

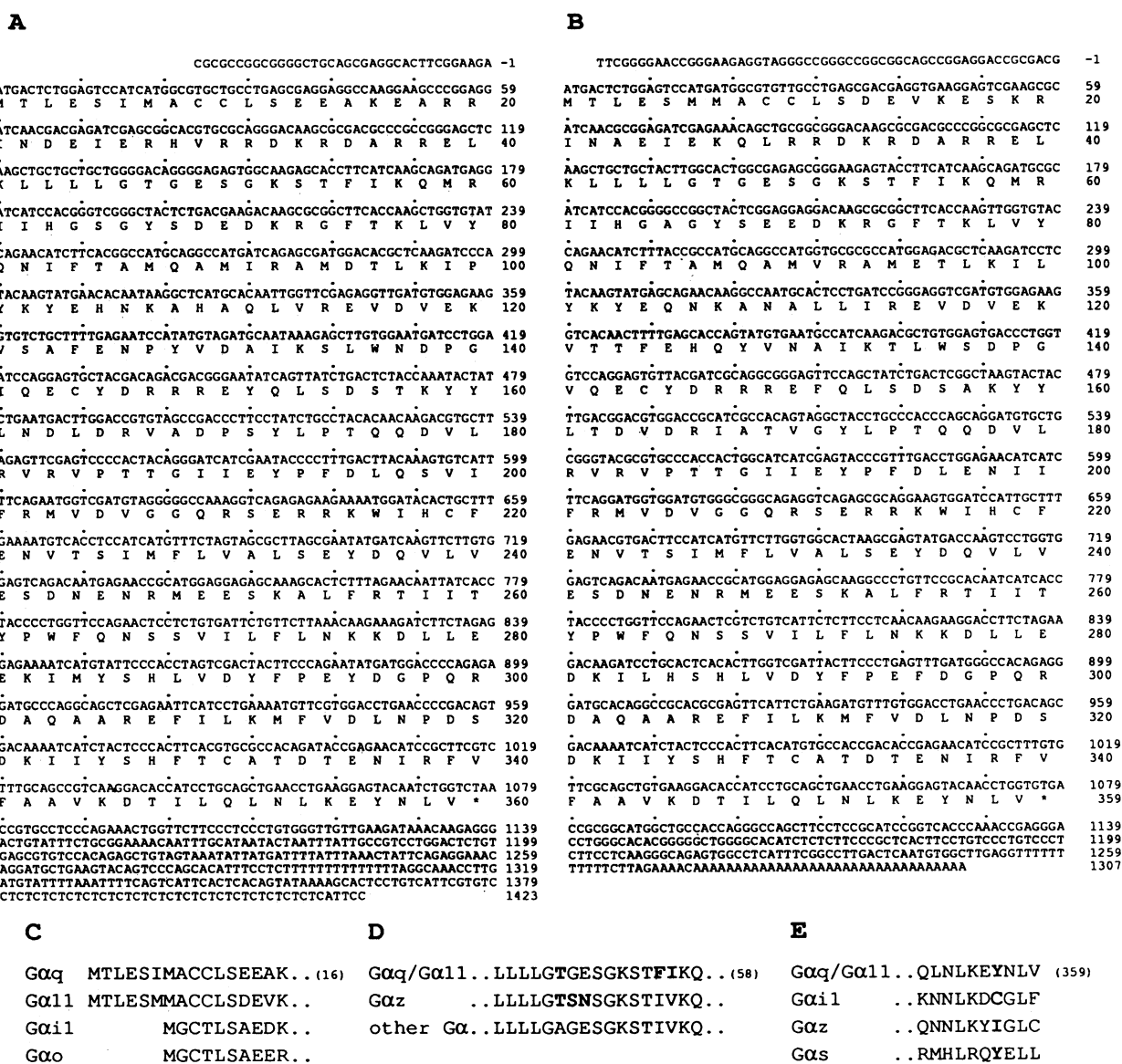


FIG. 1. Sequence of $G\alpha_q$ and $G\alpha_{11}$. The sequence of $G\alpha_q$ (A) was obtained from two clones, Gq3 and Gq7. These clones were isolated from a random-primed mouse brain cDNA library by using G69 as a probe. These clones contain the following nucleotides: Gq3, -36 to 966; Gq7, 144 to 1423; and G69, 120 to 629. The $G\alpha_{11}$ sequence (B) is contained in two clones, G23 and G11-5'-9. G23, which extends from nucleotide 490 to nucleotide 1307, was isolated from an oligo(dT)-primed mouse brain cDNA library by using the $G\alpha_{11}$ PCR fragment (15) as a probe. G11-5'-9 was isolated from the random-primed library mentioned above. This clone extends from nucleotide -56 to nucleotide 957. The N termini (C), GTP-binding domains (D, ref. 2), and the C termini (E) of several α subunits are compared. The position of the last amino acid in each domain of $G\alpha_n$ is given in parenthesis.

bined in equimolar ratios and labeled by random priming as described (15). This probe was hybridized to the filters at 50°C in 0.90 M NaCl/6 mM EDTA/60 mM NaH₂PO₄ (6× SSPE)/0.1% SDS/5× Denhardt's solution containing denatured salmon sperm DNA at 100 µg/ml (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone). The filters were washed in 6× SSC/0.1% SDS three times for 5 min at room temperature and once at 50°C for 15 min. To obtain the entire sequence of $G\alpha_q$ and $G\alpha_{11}$, a random hexanucleotide or oligo(dT)-primed mouse brain cDNA library in λ JSM8 or λ ZAPII (Stratagene) was screened by using standard techniques. $DG\alpha_q$ was cloned from a *Drosophila melanogaster* head-specific library (see Results) kindly provided by B. Hamilton (Biology Division, California Institute of Technology, Pasadena, CA).

RESULTS

Nucleotide Sequence of $G\alpha_q$ and $G\alpha_{11}$. The clone G69, which encodes part of $G\alpha_q$, was isolated from a mouse brain cDNA library by low-stringency hybridization. It cross-hybridized to a probe consisting of a mixture of cDNAs encoding all the previously known G protein α subunits (see Materials and Methods). Further screening yielded overlapping cDNA clones containing the entire $G\alpha_q$ coding sequence (Fig. 1A). The cloned PCR product corresponding to $G\alpha_{11}$, which included 180 base pairs of sequence (15), was used as a probe to screen a mouse brain cDNA library. Several clones were purified and sequenced. Fig. 1B shows the sequence of $G\alpha_{11}$ derived from overlapping cDNA fragments. The deduced amino acid sequence of $G\alpha_{11}$ is 88% identical to that of $G\alpha_q$. Almost all of the amino acid differences between $G\alpha_q$ and $G\alpha_{11}$ are concentrated in the N-terminal half of the protein. Of 42 amino acid differences, 38 are found in the N-terminal region composed of amino acids 1–200, while there are only 4 amino acid changes in a stretch of polypeptide encompassed by amino acids 201–359.

When $G\alpha_q$ and $G\alpha_{11}$ are compared with the other α subunits, a number of noteworthy differences emerge. Fig. 1C shows a comparison of the N-terminal sequences of $G\alpha_q$ and $G\alpha_{11}$ with those found for other α subunits. The methionine predicted by homology to be the first codon in $G\alpha_q$ and $G\alpha_{11}$ is preceded by other methionines in frame. The six additional amino acids found in these two α subunits are highly conserved, suggesting that they are functionally significant. The nucleotide sequences of the two cDNAs diverge upstream of these codons, indicating that there may be no further extension of this reading frame. Also in this 5' region, the $G\alpha_{11}$ cDNA contains a stop codon in frame with the downstream coding sequence.

The N termini of some α subunits are N-myristoylated on a glycine at the second position (Gly-2) (17). On the basis of their deduced amino acid sequences, $G\alpha_q$ and $G\alpha_{11}$ are not substrates for myristoylation (Fig. 1C); this may affect their membrane association properties. However, $G\alpha_s$ is not myristoylated, yet this α subunit is membrane associated. Perhaps other forms of post-translational modification will prove to be responsible for anchoring these hydrophilic proteins to the membrane.

Fig. 1D compares the amino acid sequences in the region of the "GAGE-box." This domain is highly conserved among all α subunits and has been implicated in GTP binding. Mutations in this region affect the GTPase activity of the α subunit (18, 19). $G\alpha_z$ [also named $G\alpha_x$ (20, 21)] differs in this region from the other α subunits. The slow rates of guanine nucleotide exchange and GTP hydrolysis exhibited by $G\alpha_z$ may be due in part to these sequence changes (22). $G\alpha_q$ and $G\alpha_{11}$ show differences in this region as well (Fig. 1D). Consequently, these α subunits may display unusual kinetic

properties when the proteins are characterized (see Discussion).

The C-terminal region of the α subunit is of considerable interest. The cysteine residue that lies four amino acids from the end of most mammalian α subunits can be ADP-ribosylated by pertussis toxin, thereby inactivating the G protein. Among the known α subunits, only $G\alpha_s$, $G\alpha_{olf}$, and $G\alpha_z$ lack this cysteine. These proteins are refractory to modification by pertussis toxin (2, 22). Fig. 1E shows that $G\alpha_q$ and $G\alpha_{11}$ also lack the cysteine residue at this position. Thus it is likely that the proteins corresponding to these cDNA clones will be insensitive to pertussis toxin modification.

Distribution of $G\alpha_q$ and $G\alpha_{11}$. To determine the relative size of the mRNAs corresponding to $G\alpha_q$ and $G\alpha_{11}$ and their distributions, Northern hybridization was performed with RNA purified from a variety of mouse tissues. Both $G\alpha_q$ and $G\alpha_{11}$ are ubiquitously expressed (Fig. 2). Using a probe specific to the 3' untranslated region of each cDNA clone, we found that $G\alpha_q$ has multiple messages, whereas $G\alpha_{11}$ shows a single band of approximately 4 kb. The three largest $G\alpha_q$ transcripts (approximately 5, 6, and 7.5 kb) were evident (Fig. 2A) in other RNA preparations, suggesting that they are not the products of a single degraded message (data not shown). The very large size of the $G\alpha_q$ message and the variation in signal strength in some of the tissues led us to use another method to probe for the presence of $G\alpha_q$ and $G\alpha_{11}$ specific RNA. This technique, termed PCR Northern analysis (T. M. Wilkie and M.I.S., unpublished), utilizes oligonucleotide primers designed to amplify a pool of α subunits by PCR. Specific sequences in the pool are detected by oligonucleotide hybridization. The $G\alpha_{11}$ message (Fig. 2C) was found to be expressed in all tissues that were examined. The $G\alpha_q$ message (Fig. 2C) was also found in all tissues, although the relative levels appeared to be lower in RNA samples derived from intestine and testes and higher in brain and lung.

Analysis of the Relationships Among the G Protein α Subunits. Itoh *et al.* (23) have described the relationships between the α subunits on the basis of amino acid identity. These comparisons suggest evolutionary relationships that may exist among the G proteins. An expansion of this analysis

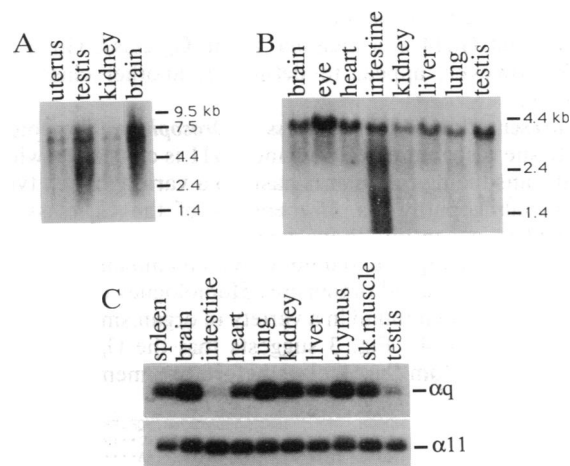


FIG. 2. Northern and PCR Northern analysis of $G\alpha_q$ and $G\alpha_{11}$. Probes specific to $G\alpha_q$ (A) and $G\alpha_{11}$ (B) were hybridized to poly(A)⁺ RNA (A) and total RNA (B) from various mouse tissues. Loads were 20 µg of total RNA per lane and 4 µg of poly(A)⁺ RNA per lane; however, ethidium bromide staining revealed that the amount of ribosomal RNA contamination varied among the different tissues. kb, Kilobases. PCR Northern analysis (C) was performed on various mouse tissues by using the degenerate oligonucleotides oMP19, oMP20, and oMP21. The amplified products were hybridized with radiolabeled oligonucleotides specific to $G\alpha_q$ and $G\alpha_{11}$.

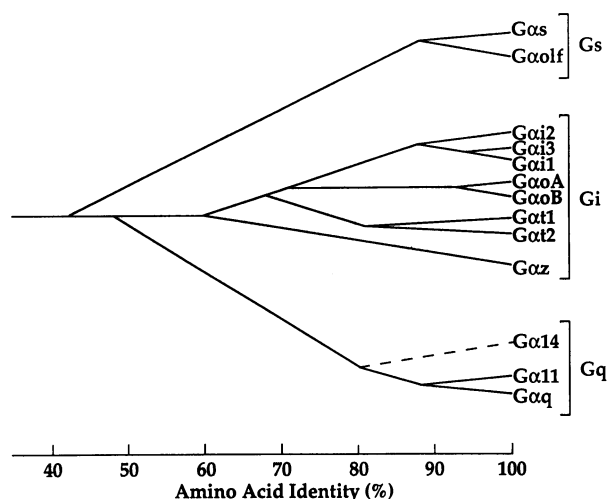


FIG. 3. Relationships among mammalian G protein α subunits. The α subunits are grouped by amino acid identity (2). Branch junctions approximate values calculated for each pair of sequences. $G\alpha_oA$ and $G\alpha_oB$ represent splice variants of the $G\alpha_o$ gene (24, 25). A broken line extends from $G\alpha_{14}$ because the entire sequence of this α subunit is not available.

reveals three classes of α subunits: G_s , G_i , and G_q (Fig. 3). Within a class, α subunits display not only primary sequence relationships but also, to some extent, functional similarities. Thus in reconstitution studies some α subunits show significant levels of "crosstalk." For example, $G\alpha_{olf}$ can substitute for $G\alpha_s$ in coupling the β -adrenergic receptor to adenylyl cyclase (26). Also, the three $G\alpha_i$ subtypes open atrial potassium channels (27). The G_i class includes all the known α subunits that are susceptible to pertussis toxin modification.

$G\alpha_q$ and $G\alpha_{11}$ form a separate class (Fig. 3). They show less than 50% amino acid sequence identity with any of the other α subunits. It is likely that $G\alpha_q$ and $G\alpha_{11}$ will display significant crosstalk, since they differ at only four residues over the C-terminal 144 amino acids. This C-terminal domain is thought to contain the structural elements required for specific interactions with effector and receptor (28). Comparison of these amino acid sequences with the partial sequences uncovered in the PCR screen described earlier (15) reveals that $G\alpha_{14}$ is a member of the G_q class. $G\alpha_{12}$ and $G\alpha_{13}$, however, appear to define yet another class of α subunits.

Representation of the G_q Class in *Drosophila*. The ubiquitous tissue distribution of $G\alpha_q$ and $G\alpha_{11}$ is consistent with a signal transducing role that is basic to a variety of cell types and signaling pathways. If members of the G_q class are involved in a central "housekeeping" pathway, then this class should be represented not only in mammals but also in other distantly related organisms. Homologues of $G\alpha_s$, $G\alpha_i$, and $G\alpha_o$ have been found in a variety of organisms, including *Drosophila* (29–34). Fig. 3 suggests that the G_q class may have diverged from the G_i class before two members of the

G_i class, G_o and $G\alpha_i$, diverged from each other. Thus it seemed possible that *Drosophila* would have a $G\alpha_q$ homologue. These arguments encouraged us to screen *D. melanogaster* α subunits for a member of the G_q class. We used the same PCR technique described previously (15) with mixed degenerate oligonucleotides corresponding to two highly conserved amino acid hexamer sequences found in all of the G proteins thus far studied. We screened clones derived from amplification of RNA isolated from 0- to 24-hr embryos of *Drosophila* and from total *Drosophila* adult RNA. We initially obtained a PCR product from both RNA sources which when sequenced differed from the murine $G\alpha_q$ at 3 of the deduced 51 amino acids. This PCR product was used to probe a *Drosophila* head cDNA library, and a clone was isolated and sequenced. The deduced amino acid sequence of the *Drosophila* $G\alpha_q$ homologue ($DG\alpha_q$) is compared with $G\alpha_q$ and $G\alpha_{11}$ in Fig. 4. $DG\alpha_q$ is 76% identical (88% similar) to $G\alpha_q$. This α subunit lacks the putative N-terminal six additional amino acids; however, it has the characteristic changes in the sequence of the GTP-binding domain (GAGE box, Fig. 1D). In addition, $DG\alpha_q$ is predicted to be insensitive to pertussis toxin on the basis of its C-terminal amino acid sequence.

DISCUSSION

The analysis of cDNA clones encoding $G\alpha_q$ and $G\alpha_{11}$ demonstrates the existence of ubiquitously expressed highly homologous G protein α subunits. During the characterization of these cDNA clones, Pang and Sternweiss used affinity chromatography with immobilized $\beta\gamma$ subunits (35) to purify novel α subunits (36). They found α subunits with an apparent molecular mass of 42 kDa. The amino acid sequences of tryptic fragments from these proteins were identical to the deduced amino acid sequence of $G\alpha_q$. One of the peptides also showed a sequence identical to that of $G\alpha_{11}$. Furthermore, Pang and Sternweiss (36) found that the 42-kDa proteins have the properties that we would ascribe to $G\alpha_q$ and $G\alpha_{11}$; they are not substrates for pertussis toxin modification, and they have unusual nucleotide binding kinetics. Using peptide antisera, they showed that the proteins are abundant in brain and lung extracts and present at lower levels in a variety of tissues.

There are many examples in the literature of effects of GTP and GTP analogs on signal transducing processes that are difficult to account for on the basis of the previously characterized G protein α subunits. Perhaps the most prominent among these is the observation that in many systems inositol trisphosphate and diacylglycerol release mediated by G protein activation of phospholipase C is pertussis toxin resistant (37). $G\alpha_z$ is thought to be a candidate for the G protein that might mediate pertussis toxin-insensitive activation of phospholipase C. However, $G\alpha_z$ expression appears to be restricted to neural tissues and platelets and it does not appear to be present in all tissues that show pertussis toxin-insensitive activation of phospholipase C (38). On the other hand, $G\alpha_q$ and $G\alpha_{11}$ are ubiquitously expressed. Hence they

DG α_q	MECCLEEAEKQKRNQIEKQLRRDKRDARRELKLLLTGSGSKSTFIKQMRIHSGSYSDKRGYIKLVFQNIFFMAMQSM	84
G α_q	MTLESI A.....AR.....D.....RHV.....FT.....T.....A.....	90
G α_{11}	MTLESM A.....D.....V.....S.....A.....E.....FT.....T.....A.....	90
	IKAMMLKISYGGQGHSELADLVMSIDYETVTTTFEDPYLNAIKTLMDAGIQECYDRRREYQLTDSAKYYLSDLARIEQADYLPEQDIL	174
	R.....T.....P.....KYEHKNAH.....Q.....REV.....V.....K.....SA.....N.....VD.....S.....N.....P.....S.....T.....N.....D.....VADPS.....Q.....V.....	180
	VR.....ET.....L.....KYEQNKAN.....L.....IREV.....V.....K.....HQ.....V.....S.....P.....V.....F.....S.....T.....VD.....ATVG.....Q.....V.....	180
	RARVPTTGILEYFPDLDGIVFRMVDVGGQRSEKRWIHCENVTSSIIIFLVALSEYDQILFSDNENRMEESKALFRTIITYPWFQNSVI	264
	V.....I.....QSVI.....M.....V.....V.....	270
	V.....I.....EN.....I.....M.....V.....V.....	270
	LFLNKKDLLEEKIMYSHLDYFPEYDGPQKQDHAAKQFVLKKYLACNPDPERQCYSHFTTATDTENIKLVCAVKDITMKNALKEFNLG	353
Q.....R.....A.....Q.....R.....E.....I.....MEVDL.....SDKII.....C.....R.....F.....A.....L.....LN.....Y.....V.....	359
D.....LH.....F.....Q.....R.....A.....Q.....R.....E.....I.....MEVDL.....SDKII.....C.....R.....F.....A.....L.....LN.....Y.....V.....	359

FIG. 4. Amino acid comparison of the *Drosophila* $G\alpha_q$ homologue ($DG\alpha_q$) with $G\alpha_q$ and $G\alpha_{11}$. Dots represent identities with the $DG\alpha_q$ sequence.

are good candidates for this role. Furthermore, there are a variety of isoforms of phospholipase C; many cells contain multiple related but distinct phospholipase C gene products (39). Members of the G_q class could be involved in the activation of specific phospholipase C isozymes. Speculation about the possible interaction of the G_q class with phospholipase C is strengthened by the recent finding that $DG\alpha_q$ message is localized mainly to the *Drosophila* eye and ocellus structures (40). Earlier work indicated that regulation of phospholipase C in invertebrate eyes is central to the phototransduction cascade (41). There are a variety of other roles that are possible for the α subunits described here—e.g., ion channel activation and phospholipase A2 activation.

cDNA clones with deduced amino acid sequences that are identical to $G\alpha_{11}$ have been found in RNA preparations from human tissue (T. T. Amatruda and M.I.S., unpublished results). The partial amino acid sequences found by Pang and Sternweiss (36) suggest that both $G\alpha_q$ and $G\alpha_{11}$ exist in rat brain extracts, and experiments with specific probes in our laboratory have detected $G\alpha_q$ and $G\alpha_{11}$ messages in a variety of tissues and cloned cell lines. These results suggest that both α subunits are expressed together in at least some cells.

The diversity of the G protein family continues to grow. As more α subunits are cloned, more classes will emerge. Within each class, highly homologous members are likely to display apparent crosstalk in reconstitution experiments. Indeed, it is difficult to discriminate between $G\alpha_{11}$ and $G\alpha_{13}$ by *in vitro* assays (27, 42). $G\alpha_q$ and $G\alpha_{11}$ may behave in similar fashion, since they are 97% identical over the domains apparently responsible for receptor and effector specificity. However, the strict evolutionary conservation of amino acid sequence differences that identify these α subunits argues that they are not redundant (2). The distinctions may become apparent as we begin to use more sophisticated assays of G protein function that include different combinations of $\beta\gamma$ dimers. There is evidence to suggest that all $\beta\gamma$ subunits are not equivalent (43, 44). Perhaps the diversity afforded by combinatorial associations of α , β , and γ subunits allows a cell to "fine tune" G protein function to the specific requirements of a particular signaling response.

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